3 '-processing of yeast tRNA(Trp) precedes 5 '-processing

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ABSTRACT

Previous analyses of eukaryotic pre-tRNAs processing have reported that 5′-cleavage by RNase P precedes 3′-maturation. Here we report that in contrast to all other yeast tRNAs analyzed to date, tRNA^{Trp} undergoes 3′-maturation prior to 5′-cleavage. Despite its unusual processing pathway, pre-tRNA^{Trp} resembles other pre-tRNAs, showing dependence on the essential Lsm proteins for normal processing and efficient association with the yeast La homolog, Lhp1p. tRNA^{Trp} is also unusual in not requiring Lhp1p for 3′ processing and stability. However, other Lhp1p-independent tRNAs, tRNA$_2^{Lys}$ and tRNA$_1^{Ile}$, follow the normal pathway of 5′-processing prior to 3-processing.

Keywords: tRNA processing; La protein; Lsm proteins; *Saccharomyces cerevisiae*

INTRODUCTION

Eukaryotic tRNAs are synthesized as precursors that undergo posttranscriptional maturation, including 5′- and 3′-processing, addition of the 3′-terminal-CCA$_{OH}$ sequence, and numerous nucleoside modifications. In addition, several pre-tRNAs contain introns that are removed by specific RNA splicing involving cleavage and religation. In both prokaryotes and eukaryotes, maturation of the 5′-end is performed by endonuclease RNase P (for review, see Altman et al. 1995; Frank and Pace 1998). Processing of the 3′ terminus of bacterial tRNAs is exonucleolytic (Li and Deutscher 1996), whereas in eukaryotes, the major pathway involves an endonucleolytic cleavage (Garber and Altman 1979; Hagenbüchle et al. 1979; Castaño et al. 1985; Frendewey et al. 1985; Manam and Van Tuyle 1987; Chen and Martin 1988; Furter et al. 1992; Oommen et al. 1992; Han and Kang 1997; Nashimoto 1997; Kunzmann et al. 1998; Mayer et al. 2000; Schiffer et al. 2002), with exonucleolytic trimming as an alternative pathway (Garber and Altman 1979; Solari and Deutscher 1983; Engelke et al. 1985).

In the budding yeast *Saccharomyces cerevisiae*, in vivo analysis of pre-tRNA processing revealed endonucleolytic 3′-processing only after 5′-processing (Engelke et al. 1985; O'Connor and Peebles 1991; Furter et al. 1992). This was confirmed by analysis of mutants defective in RNase P. Mutations in either the RNA or protein components of RNase P inhibited both 5′- and 3′-processing of pre-tRNA in vivo, even though the enzyme has no known direct role in 3′-processing (Lee et al. 1991; Lygerou et al. 1994). In contrast, end maturation and splicing occur independently (O'Connor and Peebles 1991), but usually the rate of end maturation is faster and tRNA processing intermediates include high levels of the unspliced but end-matured pre-tRNAs. In other eukaryotes, reports of 3′-processing preceding 5′-processing are rare but not unknown. For example, this processing order has been reported for silkworm and wheat germ extracts (Garber and Gage 1979; Arends and Schön 1997) and for some tRNAs in mouse (Rooney and Harding 1986).

Endonucleolytic 3′-cleavage, by an as yet unidentified enzyme, is stimulated by yeast homolog of human La phosphoprotein Lhp1p (La-homologous protein; Van Horn et al. 1997; Yoo and Wolin 1997). Lhp1p is nonessential for viability, but its binding to 3′ poly(U) tracts in pre-tRNAs protects RNA against degradation and suppresses 3′-maturation by exonucleases. In *lhp1*-Δ cells, mature 3′-ends of tRNAs are synthesized by exonucleolytic trimming, and this generates characteristic pattern of pre-tRNA intermediates for most tRNAs analyzed (Yoo and Wolin 1997). Normal tRNA processing and efficient association of pre-tRNAs with Lhp1p requires also the presence of seven Lsm proteins, Lsm2–8p (Like-Sm; Kufel et al. 2002). Lsm2–8p exist in a heptameric complex present in the nucleus, are core proteins of U6 snRNP essential for U6 stability, and are involved in pre-mRNA splicing (Cooper et al. 1995; Séraphin, 1995; Pannone et al. 1998; Achsel et al. 1999,

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Article and publication are at http://www.rnajournal.org/cgi/doi/10.1261/rna.2145103.

2001; Gottschalk et al. 1999; Mayes et al. 1999; Salgado-Garrido et al. 1999; Stevens and Abelson 1999; Collins et al. 2001). Lsm proteins have been shown to transiently associate with pre-tRNAs, and this interaction probably facilitate their binding to Lhp1p. Accordingly, the absence of the essential proteins, Lsm2–5p or Lsm8p, leads to strong accumulation of pre-tRNA processing intermediates (Kufel et al. 2002).

We examined the order of processing for three pre-tRNA species that do not show perturbed 3′-processing in the absence of Lhp1p (Yoo and Wolin 1997). Pre-tRNA$_2^{Lys}$ and pre-tRNA$_1^{Ile}$, conformed to the normal pattern, with 5′-processing preceding 3′-processing. However, pre-tRNA^{Trp} was conspicuously different, with the 5′-unprocessed, 3′-processed pre-tRNA readily visible.

RESULTS

Pre-tRNA^{Trp} is unusual in being 3′-processed prior to 5′-processing

For all pre-tRNAs, several processing intermediates are readily visible in wild-type cells. In the case of the well-studied species tRNA$_3^{Leu}$, a probe that hybridizes to the 3′-spacer region (probe 317; Fig. 1O,P) detects the primary transcript and the spliced, 5′- and 3′-unprocessed intermediate, as well as the 5′-processed, 3′-unprocessed pre-tRNA. In contrast, a probe that hybridizes to the 5′-leader (probe 315; Fig. 1Q,R) detects only the 5′- and 3′-unprocessed species. A probe specific for the intron sequence (probe 307; Fig. 1S) also detects the 5′-processed, 3′-unprocessed pre-tRNA, but no 3′-processed, 5′-unprocessed species. This is the expected consequence of 5′-processing by RNase P prior to tRNA 3′-maturation. Inhibition of RNase P activity in a temperature-sensitive *rpr1-1* strain (Fig. 1, lanes 3–7) results in accumulation of the 5′- and 3′-unprocessed species, showing that the inhibition of 5′-processing also prevents 3′-processing (Lee et al. 1991).

In an *lhp1*-Δ strain (Fig. 1, lane 9) the primary transcript of tRNA$_3^{Leu}$ and most other tRNAs becomes heterogeneous owing to 3′-truncation, and the 5′-processed, 3′-unprocessed intermediate is lost (Fig 1M, lane 9; Yoo and Wolin 1997). This reflects the inhibition of 3′-endonuclease cleavage and the activity of 3′ → 5′ exonucleases in the absence of Lhp1p binding. However, for three species tested—tRNA^{Trp}, tRNA$_2^{Lys}$, and tRNA$_1^{Ile}$—the 5′-processed, 3′-unprocessed pre-tRNAs were unchanged in *lhp1*-Δ cells (shown for tRNA^{Trp} and tRNA$_2^{Lys}$ in Fig. 1G,M, respectively; Yoo and Wolin 1997). The order of processing of these tRNAs was analyzed in more detail by Northern hybridization. Data is shown for tRNA^{Trp} and tRNA$_2^{Lys}$ in Figure 1; processing of tRNA$_1^{Ile}$ was very similar to that of tRNA$_2^{Lys}$. Processing of tRNA$_2^{Lys}$ (Fig. 1I–N) and tRNA$_1^{Ile}$ (data not shown) followed the normal pathway, with 5′-cleavage preceding 3′-processing. However, the intermediates detected for tRNA^{Trp} were clearly different (Fig. 1A–H).

Five different genes encode pre-tRNA^{Trp} species, which have identical introns and mature tRNA sequences (there is only one Trp anticodon) but differ in their 5′- and 3′-spacer regions (see Fig. 1V). Oligonucleotides specific for the 3′-regions of pre-tRNA$_{VII}^{Trp}$ (oligo 318; roman numerals denote the chromosomal location of the gene encoding a particular tRNA^{Trp}) and pre-tRNA$_X^{Trp}$ (oligo 316) detected only the full-length pre-tRNA primary transcript in wild-type cells (Fig. 1A,D, lanes 1,2,8). In contrast, oligonucleotides specific for the 5′-regions of pre-tRNA$_{VII}^{Trp}$ (oligo 314) and pre-tRNA$_X^{Trp}$ (oligo 319) detected both the primary transcripts and shorter species (Fig. 1B,F). These RNAs were not detected by 3′-specific probes, indicating that the pre-tRNAs had been 3′-processed without prior 5′-maturation. 5′-extended forms were seen for both spliced and unspliced pre-tRNA^{Trp} (Fig. 1C,F) indicating that as for other pre-tRNAs, end maturation is independent of splicing. A probe against mature tRNA^{Trp} (oligo 305; Fig. 1G) detected the same pre-tRNAs as the 5′-specific probe, indicating that this pattern of processing is common to the tRNA^{Trp} gene family. For many yeast tRNA species, minor differences in the pattern of processing intermediates can be seen in comparisons of cells grown at 25°C and 37°C (O'Connor and Peebles 1991; Kufel et al. 2002) and this is also the case for tRNA^{Trp}.

The identification of the pre-tRNA^{Trp} species with 5′-leaders and mature 3′-ends was supported by their persistence in *lhp1*-Δ strain (Fig. 1B,E,G, lane 9), because tRNA precursors with mature 5′-ends and 3′-extensions are generally depleted in strains lacking Lhp1p (see pre-tRNA$_3^{Leu}$ in Fig. 1S; Yoo and Wolin 1997).

After transfer of the *rpr1-1* strain to 37°C to inhibit 5′-processing, the primary transcripts of tRNA$_2^{Lys}$ was accumulated, as well as the 3′- and 5′-unprocessed spliced forms (Fig. 1I–M, lanes 3–7). The unspliced pre-tRNA forms that were 5′-processed, 3′-unprocessed, or both 5′- and 3′-processed are depleted (Fig. 1I,M). As seen for pre-tRNA$_3^{Leu}$, 3′-processing is blocked by the inhibition of 5′-processing. A quite different picture was seen for the tRNA^{Trp} species. Here the inhibition of 5′-processing also caused depletion of 5′-processed forms (Fig. 1G) and accumulation of primary transcripts (Fig. 1A,B,D,E,G). In marked contrast, the unspliced and 5′-unprocessed but 3′-processed pre-tRNA$_{VII}^{Trp}$ accumulated to high levels (Fig. 1B), as did the spliced, 5′-unprocessed but 3′-processed form of both pre-tRNA$_{VII}^{Trp}$ and tRNA$_X^{Trp}$ (Fig. 1C,F). The inhibition of 5′-processing did not, therefore, prevent 3′-processing of pre-rRNA^{Trp}, in contrast to all other tRNAs tested.

Accumulation of different levels of processing intermediates for pre-tRNA$_{VII}^{Trp}$ and tRNA$_X^{Trp}$ (see Fig. 1B,E) indicate that even in the same tRNA gene family, the preferential order of end maturation and splicing may differ.

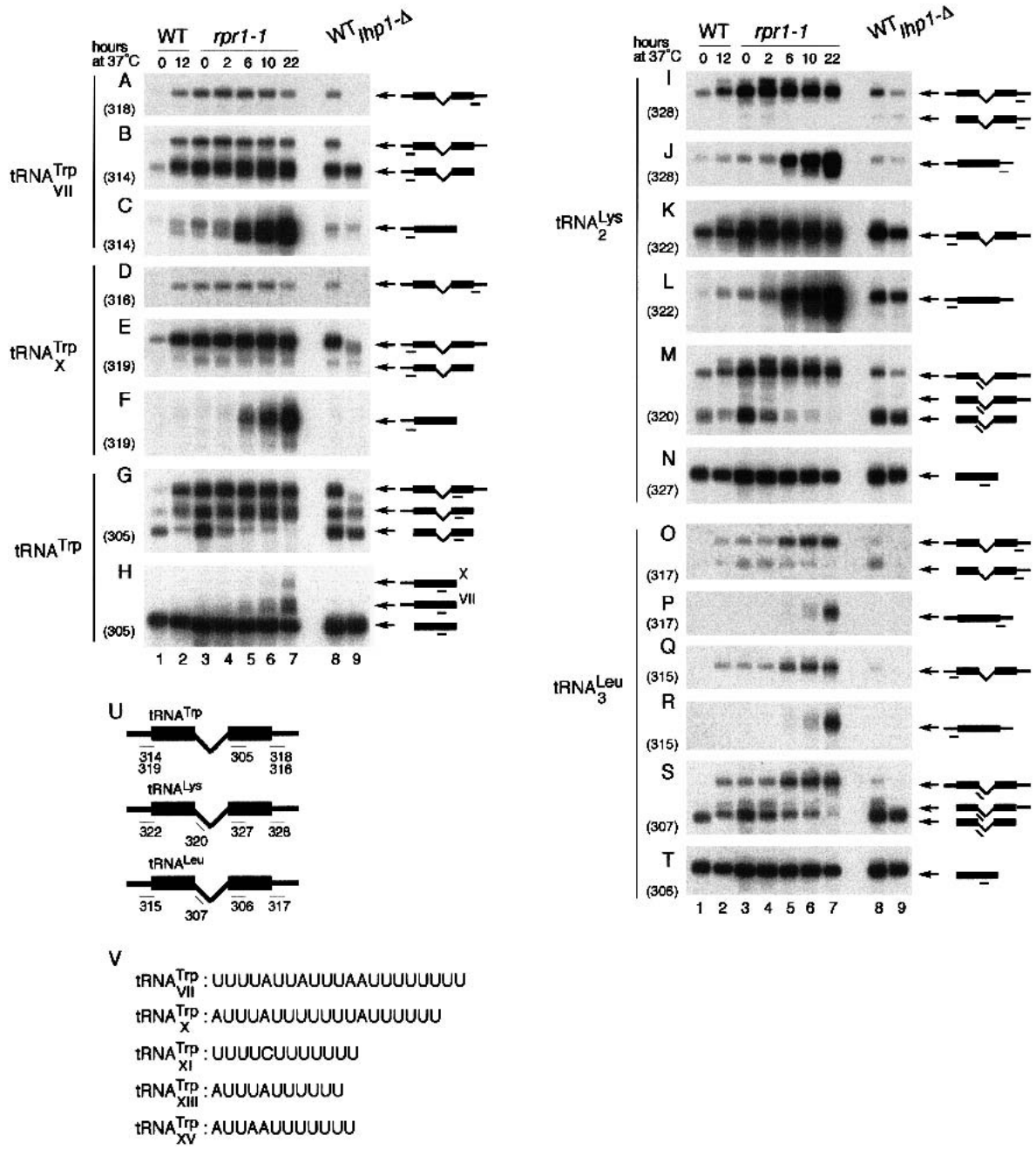

FIGURE 1. Processing of pre-tRNA^{Trp}. (*A–T*) RNA was extracted from wild-type cells grown at 23°C (lane *1*) or 12 h after transfer to 37°C (lane *2*); from the otherwise isogenic *rpr1-1* strain grown at 23°C (lane *3*) or after transfer to 37°C for 2, 6, 10, and 22 h (lanes *4–7*); and from otherwise isogenic wild-type and *lhp1*-Δ strains grown at 30°C (lanes *8,9*). Names of tRNAs are on the *left*, schematic representations of tRNA precursors, intermediates, and mature species are shown on the *right*. Probe names are shown in parentheses, and their locations are indicated as lines under the schematics of the pre-tRNA species and in panel *U*. (*V*) Sequences immediately downstream of the 3′-mature ends of the five pre-tRNA^{Trp} species.

3′-processed pre-tRNA^{Trp} shows Lsm-dependence for normal processing and efficient association with Lhp1p

Lsm proteins are required for the normal processing of all pre-tRNAs analyzed (Kufel et al. 2002) (see Fig. 2A,VI for tRNA$_3^{Leu3}$). Genetic depletion of Lsm2 to 5p or Lsm8p inhibited processing of pre-tRNA^{Trp} (shown for Lsm2p and Lsm3p in Fig. 2A). Similar phenotypes were also observed in temperature-sensitive (ts) *lsm2*^{ts} or *lsm5*^{ts} strains (Fig. 2B), in which fusion constructs between the DNA binding domain of Gal4p and Lsm2p and Lsm5p, respectively, are expressed in *lsm2*-Δ and *lsm5*-Δ backgrounds (Fromont-Racine et al. 2000; Tharun et al. 2000; He and Parker 2001). The primary transcript was strongly accumulated, as were

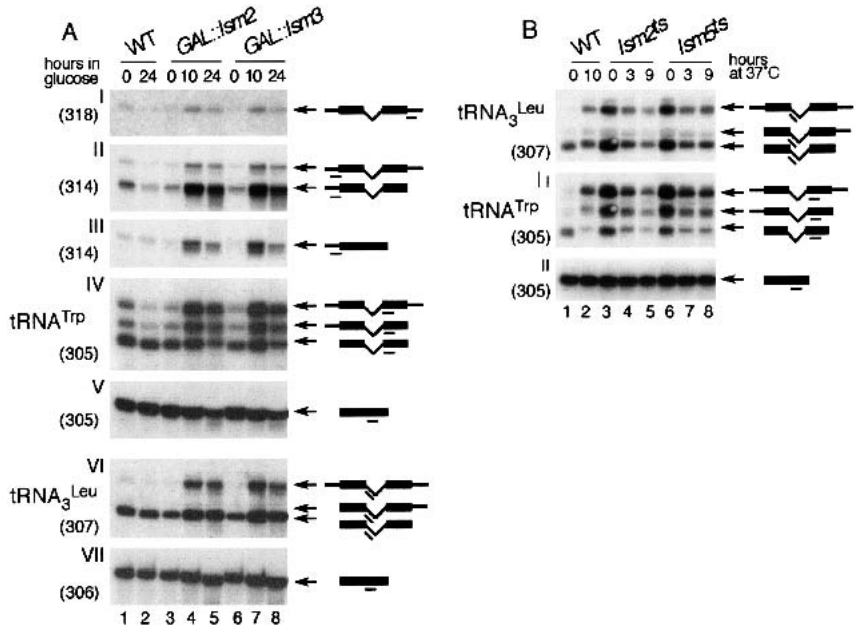

FIGURE 2. Normal processing of pre-tRNA^{Trp} requires Lsm proteins. Processing of pre-tRNA^{Trp} and pre-tRNA$_3^{Leu}$ in *GAL-lsm* (*A*) and *lsm^{ts}* (*B*) strains. Strains carrying *GAL*-regulated constructs (*GAL::lsm*, *A*, lanes *3–8*) and the BMA64 wild-type strain (WT, *A*, lanes *1,2*) were grown in permissive RSG medium (0 h) and transferred to repressive glucose medium at 30° for the times indicated. The temperature-sensitive *lsm2^{ts}* and *lsm5^{ts}* strains (*B*, lanes *3–8*) and the wild-type strain (WT, *A*, lanes *1,2*) were pregrown at 23°C (0 h) and transferred to 37°C for the times indicated. RNA was separated on a 6% polyacrylamide gel and hybridized with oligonucleotide probes.

the spliced and unspliced forms of the 3′-processed, 5′-unprocessed pre-tRNA. In this respect the spliced, 3′-processed pre-tRNA^{Trp} differs from the spliced, 5′-processed forms of other pre-tRNAs, which are not accumulated on Lsm-depletion (Fig. 2A, VI; data not shown). This indicates that 5′-processing of tRNA^{Trp} by RNase P is stimulated by the Lsm complex. Strong pre-tRNA accumulation was also observed 6 h after transfer to glucose in *GAL-lsm* strains (data not shown), prior to the time at which effects on growth are detected (Mayes et al. 1999).

tRNA precursors associate with Lhp1p (Yoo and Wolin 1997), and this interaction is decreased after depletion of Lsm3p (Kufel et al. 2002). The primary transcript of pre-tRNA$_{VII}^{Trp}$ was co-precipitated with Lhp1-ProtA (Fig. 3A, II), and the unspliced but 3′-processed form was also precipitated, although with lower efficiency (see Table 1). In contrast, the spliced, 3′-processed pre-tRNA$_{VII}^{Trp}$ was not detectably co-precipitated (Fig. 3A, III). Similar results were obtained by using the mature probe to look at all tRNA^{Trp} species (Fig. 3A, IV), with strong precipitation of the primary transcript and weaker but clear precipitation of the unspliced but 3′-processed form. As expected, no precipitation was seen for the mature tRNA^{Trp} (Fig. 3A, V).

Depletion of Lsm3p from a strain expressing Lhp1p-ProtA resulted in reduced precipitation of the primary transcript and the loss of detectable precipitation of the 3′-processed, 5′-unprocessed forms of all pre-tRNA^{Trp} species (Fig. 3A, II and IV, lane 12; Fig. 3B; Table 1).

We conclude that despite the difference in processing pathway, pre-tRNA^{Trp} resembles other pre-tRNAs, in its dependence on the essential Lsm proteins for normal processing and efficient association with Lhp1p.

DISCUSSION

All previous analyses of tRNA processing in yeast have concluded that 5′-end maturation obligatorily precedes 3′-maturation (Engelke et al. 1985; Lee et al. 1991; O'Connor and Peebles 1991; Furter et al. 1992; Lygerou et al. 1994). In contrast to other pre-tRNAs, 5′-unprocessed, 3′-processed forms of pre-tRNA^{Trp} were readily detected. Conversely, the 5′-processed, 3′-unprocessed intermediates, which are readily detected for other pre-tRNAs, were not observed for pre-tRNA^{Trp}. This indicated that pre-tRNA^{Trp} undergoes 3′-maturation prior to 5′-processing. This conclusion was supported by analysis of *rpr1-1* strains (Lee et al 1991), which are defective in RNase P cleavage and accumulated high levels of the 5′-unprocessed, 3′-processed tRNA^{Trp}.

For other yeast pre-tRNA species, the pattern of 5′ prior to 3′ processing is not simply owing to differences in kinetics. In strains defective for RNase P, 5′- and 3′-unprocessed pre-tRNAs accumulate to high levels (Lee et al 1991; Lygerou et al. 1994), showing that 3′ processing is prevented in the absence of prior 5′ cleavage. Previous studies have concluded that Lhp1p association with most pre-tRNAs is necessary to prevent 3′ exonuclease degradation and to promote 3′ endonuclease cleavage (Yoo & Wolin 1997). In contrast, although Lhp1p is associated with pre-tRNA^{Trp} it is not necessary for its normal processing and 3′ stability. Thus association with Lhp1p is not sufficient to impose a requirement for 5′ processing prior to 3′ processing. Analyses of strains depleted of Lsm-proteins or carrying the *lsm2^{ts}* and *lsm5^{ts}* mutations, indicate that the Lsm-complex is also not required to maintain the normal order of 5′- prior to 3′-processing for tRNAs other than tRNA^{Trp} (Kufel et al. 2002). These results indicate that either there is an as-yet-unidentified factor that binds to pre-tRNA^{Trp} and actively promotes its 3′ cleavage, or this is an intrinsic property resulting from the structure of the pre-tRNA.

tRNA^{Trp} is encoded by five different genes. Gene-specific probes demonstrate that the products of at least two of these follow this unusual processing pathway, and hybrid-

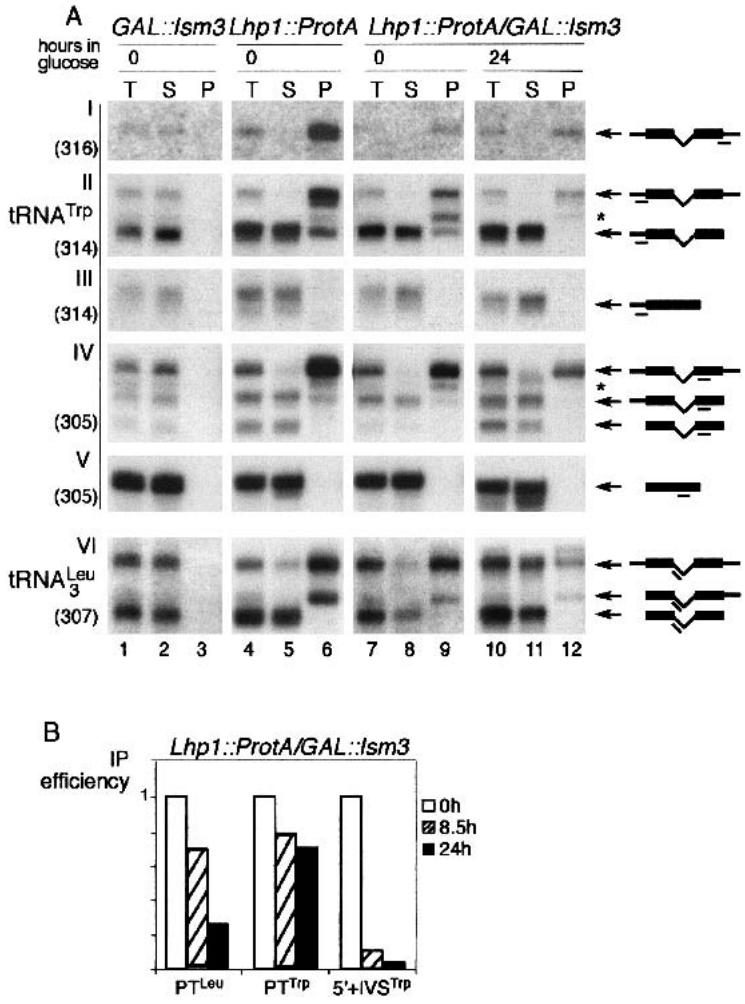

FIGURE 3. Efficient association of pre-tRNA^{Trp} with Lhp1p requires Lsm3p. (*A*) Effects of Lsm3p depletion on the association of Lhp1p with pre-tRNA^{Trp} and pre-tRNA$_3^{Leu}$. Lysates were prepared from strains *GAL::lsm3* (lanes *1–3*), *Lhp1p-ProtA* (lanes *4–6*), and *Lhp1p-ProtA*; *GAL-lsm3* (lanes *7–12*) after being grown at 30°C in RSG medium (lanes *1–9*) or after transfer to glucose medium for 24 h (lanes *10–12*) and immunoprecipitated using IgG agarose. RNA was recovered from the total lysate (T), immune supernatant (S), and the immune pellet (P) and was analyzed by Northern hybridization. Approximately fourfold more cell equivalents are loaded for the pellet fraction. The RNA band indicated with an asterisk results from degradation during immunoprecipitation. (*B*) Graphic representation of the immunoprecipitation efficiency of RNAs in the *Lhp1-ProtA/GAL-lsm3* strain in RSG medium (0 h, white bars) and after transfer to glucose medium for 8.5 h (hatched bars) and 24 h (black bars). Values for each RNA species after depletion are expressed relative to the value before depletion, which is arbitrarily set at one.

ization seen with a probe against the mature tRNA indicates that this is likely to be the case for all of the genes. However, features that might be responsible for the differences between tRNA^{Trp} and other tRNAs are not obvious. There are no clear differences in the lengths of 3′-spacer regions between pre-tRNA^{Trp} and other pre-tRNAs, and no clear complementarity between the 5′- and 3′-spacers. Some, but not all, pre-tRNA^{Trp} species have short poly(U) tracts located immediately downstream of the mature tRNA sequence (see Fig. 1, V). It is possible that these are responsible for the low level of Lhp1p precipitation seen for the 5′-unprocessed, 3′-processed pre-tRNA^{Trp}. However, longer 3′-extended species are readily observed and do not become detectably 5′-processed, in contrast to all other yeast pre-tRNAs previously analyzed.

Human La is able to recognize and bind 5′-end of tRNAs via a C-terminal Walker A motif (for review, see Maraia and Intine 2001). This interaction inhibits RNase P cleavage and is regulated by phosphorylation of La. Phosphorylation of Lhp1p is not required for its function in tRNA maturation (Long et al. 2001), and this interaction has not been shown to occur in *S. cerevisiae*, but it might contribute to the observed association of Lhp1p with the 3′-processed species.

Although tRNA^{Trp} undergoes the opposite order of 3′- and 5′-end processing than do other intron-containing tRNAs tested to date, it shares many features with other pre-tRNAs. Pre-tRNA^{Trp} binds to Lhp1p and the Lsm

TABLE 1. Immunoprecipitation efficiency by Lhp1p based on PhosphorImager quantification of Northern hybridization data from Figure 3A

Strain	GAL::lsm3	Lhp1p-ProtA	Lhp1p-ProtA/ /GAL::lsm3	Lhp1p-ProtA/ /GAL::lsm3
time in glucose (h)	t = 0 h		t = 0 h	t = 24 h
tRNA^{Trp}-PT	0	96	85	61
5′-pre-tRNA^{Trp}	0.1	17	13	0.6
tRNA$_3^{Leu}$-PT	0.9	64.2	48.4	13.1

Numbers are expressed as percent, where supernatant + pellet (P) = 100%. The value for *P* was adjusted according to the amount of loaded RNA; see legend to Figure 3.

TABLE 2. Yeast strains used in this work

Strain	Genotype	Reference/note
AEMY29	*MATα ade2-1 his3-11,-15 leu2-3,112 trp1Δ1 ura3-1 LSM5::TRP1* [pACTIIst-LSM5]	Mayes et al. 1999
AEMY30	*MATα ade2-1 his3Δ200 leu2-3,-112 trp1Δ1 ura3-1 LSM2::HIS3* [pACTIIst-LSM2]	Tharun et al. 2000
AEMY31	*MATα ade2-1 his3-11,-15 leu2-3,-112 trp1Δ1 ura3-1 LSM3::TRP1* [pBM125-GAL1-HA-LSM3]	Mayes et al. 1999
AEMY33	*MATα ade2-1 his3Δ200 leu2-3,-112 trp1Δ1 ura3-1 LSM2::HIS3* [pBM125-GAL1-LSM2-HA]	Mayes et al. 1999
YJK20	*MATa his3Δ200 leu2Δ1 trp1 ura3-52 gal2 galΔ108 LHP1::ProtA-TADH1-HIS3MX6*	Kufel et al. 2002
YJK21	as AEMY31 but *LHP1::ProtA-TADH1-HIS3MX6*	Kufel et al. 2002
YJK22	as AEMY47 but *LHP1::ProtA-TADH1-HIS3MX6*	Kufel et al. 2002
BMA64	*MATα ade2-1 his3-11,-15 leu2-3,-112 trp1Δ ura3-1*	F. Lacroute pers. comm.
YCA35	*MATa his3Δ200 leu2Δ1 trp1 ura3-52 gal2 galΔ108 LHP1::Kl URA*	Kufel et al. 1999
W3031A	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	R. Rothstein pers. comm.
rpr1-1	as W3031A but *YEp24::RPR1dim*	Lee et al. 1991

proteins (Kufel et al. 2002), and its processing and efficient association with Lhp1p requires the presence of Lsm proteins. Processing of the intron-less and dimeric classes of pre-tRNA has not yet been thoroughly analyzed, and it remains possible that other tRNA species will show the same order of processing as $tRNA^{Trp}$.

MATERIALS AND METHODS

Strains

The transformation procedure was as described (Gietz et al. 1992). Yeast strains used in this work are listed in Table 2.

RNA extraction and Northern hybridization

Growth of the *lsm* strains was as described (Kufel et al. 2002). The temperature-sensitive *rpr1-1* strain was pre-grown at 23°C and transferred to 37°C. RNA extraction and Northern hybridization were according to Tollervey (1987) and Beltrame and Tollervey (1992).

For RNA hybridization the following oligonucleotides were used: 305 ($tRNA^{Trp}$), 5′-AACCTGCAACCCTTCGA; 306 ($tRNA_3^{Leu}$), 5′-GCATCTTACGATACCTG; 307 ($tRNA_3^{Leu}$-intron), 5′-CACAGT TAACTGCGGTC; 314 (5′-$tRNA_{VII}^{Trp}$) 5′-CCACCGCTTCATCTT GAAAT; 315 (5′-$tRNA_3^{Leu}$), 5′-CCAAACAACCACTTATTTGT; 316 ($tRNA^{Trp}_X$-3′), 5′-GAAAAATAAATTGAAACGGAC; 317 ($tRNA_3^{Leu}$-3′), 5′-AAAAAATAATGGTTGCTAAG; 318 ($tRNA_{VII}^{Trp}$)-3′), 5′-TTAAATAATAAAATGAAACGGAC; 319 (5′-$tRNA_X^{Trp}$), 5′-CCACC GCTTCAACTTTTGTTG; 320 ($tRNA_2^{Lys}$-int), 5′-AACAAGGAT GAGTTCTTC; 322 (5′-$tRNA_2^{Lys}$), 5′-AACAAGGATGAGTTCTTC; 327 ($tRNA_2^{Lys}$), 5′-CTCTACCAACTGAGCTAAC; and 328 ($tRNA_2^{Lys}$-3′), 5′-AAAGTAAAGAACTCCTCATAG.

Immunoprecipitation

Whole-cell extracts were prepared as described (Séraphin and Rosbash 1989) from strains grown either in RSG medium or after the transfer to YPD medium for 8.5 or 24 h. Immunoprecipitation of *GAL::lsm3* strain and ProtA-tagged strains, *Lhp1p-ProtA*, *Lhp1p-ProtA/GAL::lsm3*, and *Lhp1p-ProtA/GAL::lsm5*, was performed as described (Lygerou et al. 1994) at 150 mM KAc. Precursors and mature RNAs were identified by Northern hybridizations.

ACKNOWLEDGMENTS

This work was supported by the Wellcome Trust.

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Received September 20, 2002; accepted October 21, 2002.

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